

CLONING EXTINCT GENES

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ABSTRACT: Genetic material from the remains of both extinct animals and ancient man has been cloned. Such research, presently in its infancy, has shown that DNA can survive for thousands of years essentially unchanged. Further research will yield valuable information regarding the genetic makeup of extinct animals, define their relationship to extant species, and answer concerns regarding the survival of infectious diseases in dead bodies.

INTRODUCTION

Under certain conditions, cytomorphologic and biochemical integrity of biological material may be preserved for long periods of time. Such material is a valuable source of knowledge about the history and development of extinct and extant organisms. Recently, several investigators have tapped this resource by investigating genetic material from extinct animals and ancient (pre-historic) man (Higuchi *et al.* 1984, Higuchi and Wilson 1984, Johnson, Olson, and Goodman 1985, Pääbo 1985). The results have been a fascinating—although rudimentary—look at ancient genetic and biochemical structure. This paper reviews the highlights of these investigations.

PALEOBIOCHEMISTRY

A full review of the field of paleobiochemistry is beyond the scope of this paper. However, it is important to recognize that paleo-DNA (deoxyribonucleic acid) studies have their roots in general paleobiochemistry and paleogenetics. The ultimate goal of most of these studies is to establish proper phylogenetic relationships between extinct and extant animal species. Analysis of traces of protein found frequently in "fossilized" specimens yields information regarding structure and function of the proteins; amino acid sequencing of these proteins also provides information on the genetic makeup of extinct animals.

However, there have been problems with this approach. First, since a significant fraction of DNA material in vertebrate cells is not transcribed or expressed (Hickey and Benkel 1986), a study of proteins as a mirror of DNA would, at best, provide only a fraction of the true genetic makeup of such animals. Further, experience has shown this approach has not been particularly successful, in part due to significant post-mortem changes in protein structure (Prager *et al.* 1980). Thus, as DNA purification and cloning technology matured, researchers sought to extract DNA material directly from the remains of extinct animals.

PALEO-DNA

Under certain conditions, cellular morphology may be preserved for thousands (Lewin 1984) or millions of years (Poinar and Hess 1982). DNA material within such specimens may survive essentially unchanged. DNA has been recovered from the quagga, an extinct equid (100 years B.P.) (Higuchi *et al.* 1984), ancient Egyptian mummies (2,400 years B.P.) (Pääbo 1985), woolly mammoth (10,000 to 53,000 years B.P.) (Johnson, Olson, and Goodman 1985), and insects preserved in amber (approximately 26 million years B.P.) (Higuchi and Wilson 1984).

The quagga (*Equus quagga*) is a zebra-like, ass-like species that became extinct in 1883 (Fig. 1). A salt-preserved skin of a specimen that died 140 years ago, stored in the Museum of Natural History at Mainz, West Germany, was the source of the genetic material isolated and cloned (Higuchi *et al.* 1984). About 0.7 g of muscle and connective tissue adherant to the animal's skin was removed and digested with proteinase K and detergent. The DNA material was purified by phenol extraction and ethanol precipitation, and then fractionated by gel-filtration and electrophoresis. This method yielded about 5 µg of DNA per g of dry tissue (about 1.5% the yield with fresh tissue). Most of the DNA segments were less than 500 base pairs long. About 10 ng of the DNA was cloned using a λ phage cloning vector, which is efficient in cloning small amounts of low molecular weight DNA.

The resultant 25,000 plaques were screened by hybridization with mitochondrial DNA (mtDNA) from the mountain zebra (*Equus zebra*). Two clones were isolated and sequenced, resulting in a total of 229 base pairs available for comparison. There were 12 base substitutions, but only two resulted in amino acid replacement. This pattern (base substitution without amino acid replacement) and degree of sequence difference (approximately 5%) is what is expected when one compares two closely related species. Further analysis allowed for calculation of the quagga-mountain zebra split to have occurred 3–4 million years ago.

Using similar techniques, Pääbo (1985) isolated and sequenced a 3.4-kilobase segment of a 2,400-year-old human DNA. Skin and subcutaneous tissues were collected from a less than one-year-old male infant mummy at the Egyptian Museum in Berlin, German Democratic Republic. The tissue was radiocarbon-dated at $2,430 \pm 120$ years B.P. Pääbo collected approximately 32 µg of DNA from 1.6 g of dry tissue—a yield of 5% that of fresh tissue. From this, he obtained a segment of 500 base pairs by using a radioactive probe with a member of the *Alu* family of repetitive human sequences. Analysis of the segment showed 77% homology to contemporary DNA—which is comparable to contemporary *Alu* repeats and indicates that there has been no significant post-mortem change over a period of 2,400 years!

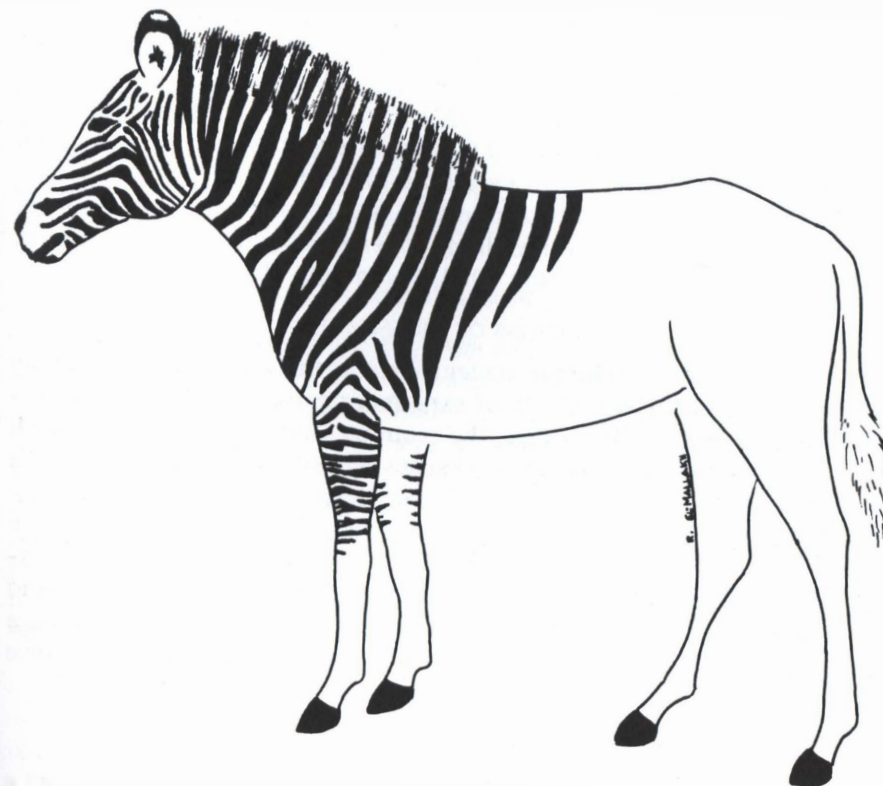


FIG. 1.—The quagga, *Equus quagga*, which is believed to have become extinct in 1883.

Older human DNA has been recovered from a peat swamp in Windover Pond, Brevard County, Florida, U.S.A. (Doran *et al.* 1986). Forty bodies of various ages were discovered in a stratum that was carbon-dated at 7,790–8,290 years B.P. Of these, five individuals had recognizable intracranial brain tissue. Histological analysis revealed remnant cellular structure. DNA was extracted with a total yield of 1 µg/g of brain tissue (1% of yield of fresh brain tissue), of which approximately 0.05% was mtDNA.

Material from two well preserved frozen woolly mammoths, *Mammuthus primigenius*, was the source of the mammoth DNA (Johnson, Olson, and Goodman 1985). Air dried muscle tissue from the Yuribei mammoth, an immature female discovered in 1979 and dated at 10,000 years old, and the Khatanga mammoth, an adult male discovered in 1978 and dated at 53,000 years old, was treated in a similar fashion as described above. The yield was variable but ranged from 8 to 38 µg DNA per g of dried tissue, or 2–12% the expected yield from fresh tissue. DNA fragments ranged from 200 to 3,000 base pairs long. These were cloned in a bacteriophage φX174. Un-

fortunately, there also appeared to be significant amounts of contaminant DNA. When the base composition of the mammoth DNA was compared with DNA extracted from the Asian elephant (*Elephas maximus*), it was found that approximately 25% of the DNA shared a similar base composition (36% G + C) (Johnson, Olson, and Goodman 1985). However, only 2–5% of the DNA was homologous in hybridization studies.

Genetic material from amber-preserved insects has not yet been cloned, but the hardness of DNA over millions of years under these conditions has been shown (Higuchi and Wilson 1984, Poinar and Hess 1982).

CONDITIONS OF PRESERVATION

The ultimate limit to cloning ancient DNA is the availability of suitably preserved material. From the brief experience gained, preliminary observations can be made. The quagga, the mummy, and amber insects were all preserved by rapid (days to a few weeks) desiccation, whereas the mammoth was preserved by freezing. DNA yields were relatively similar (in the range of 5–30 µg DNA/g dry tissue), but the mammoth DNA appeared to have a higher proportion of contaminant DNA. This was probably due to intermittent thaw and contamination by microorganisms (Johnson, Olson, and Goodman 1985). Thus, it appears that desiccated specimens may yield more precise sequence data. Further research is needed to determine the prevalence of suitable material.

RELATED RESEARCH

In Somalia, on October 26, 1977, a 23-year-old hospital cook acquired a smallpox (variola) infection (Anonymous 1980). He recovered completely, but the event is historic in that there have been no endemic cases of smallpox since. Aside from laboratory-maintained populations of smallpox, the virus has been completely eradicated from the planet. Or has it?

A recent debate has emerged in the medical literature regarding the viability of the smallpox virus (Zuckerman 1984, Lewin 1985, El-Mallakh 1985). There is evidence—both historic and experimental—that smallpox virus may remain viable in dry archaeological material (Razzel 1975, El-Mallakh 1985) or in bodies buried in permafrost (Lewin 1984, 1985). In 1759, at Chelwood, Somerset, England, the body of a man who had died 30 years earlier was exhumed in order to make room for a prominent village resident who had just died. Within several days of the exhumation, all but two people in the village were stricken with smallpox (Dixon 1985). More recently, morphologically intact but nonviable variola virions were isolated from 100-year-old bodies kept in the crypt beneath Spitalfields Church, in the east end of London (Anonymous 1985). Continuing attempts to isolate smallpox from bodies buried in permafrost have, as yet, been unsuccessful (Lewin 1985).

Although there have not been any reported cases of smallpox among archaeologists, anthropologists, or related field workers since the “eradication” of smallpox, the knowledge of long-term survival of dried DNA increases the level of concern (El-Mallakh 1985). Blanket vaccination of workers at risk has been recommended (Zuckerman 1984), but not likely to be carried out (Lewin 1985, El-Mallakh 1985). Further observation and investigation are needed to answer these concerns.

CONCLUSIONS

The recent cloning of extinct quagga and mammoth DNA and ancient human DNA raises many questions and opens many possibilities. The observation that DNA preserved under desiccating conditions survives unchanged for thousands of years will allow for more confident study of appropriately preserved specimens. Such research will improve our understanding of the proper phylogenetic relationships of extinct and extant species, which will be of significant interest to zoologists and paleontologists, as well as cryptozoologists. Implications of this research are seen in the medical field, where the long-term survival of viable variola in desiccated bodies could potentially endanger the concept of “eradication” of smallpox.

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